

The University of Chicago

TESTIS HORMONE SECRETION IN THE
RAT UNDER CONDITIONS OF
VASECTOMY OR ISOLATION

A PART OF A DISSERTATION SUBMITTED TO THE FACULTY
OF THE DIVISION OF THE BIOLOGICAL SCIENCES IN
CANDIDACY FOR THE DEGREE OF DOCTOR OF PHILOSOPHY

DEPARTMENT OF ZOOLOGY

1939

BY

HELEN POYNTER

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TESTIS HORMONE SECRETION IN THE RAT UNDER CONDITIONS OF VASECTOMY OR ISOLATION ¹

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While the capacity of the vertebrate testis to secrete hormone has been demonstrated to be directly under the control of the anterior lobe of the hypophysis (Smith, '30), experimental methods have shown that several physical and chemical factors such as light (Baker and Ranson, '32; Bissonnette, '30, '32; Rowan, '29; Benoit, '35), temperature (Wells, '35; Craig-Bennett, '31; Moore, '37), excessive alcohol intake (Arlitt and Moore, '35), vitamin deficiency (hypovitaminosis, and inanition (Mason and Samuels, '31; Evans and Burr, '33; Kudryashov, '35; Bouin, '35), and the presence of male hormone secreting cells in the anterior lobe may be directly influenced by the presence or absence of these factors.

Two other factors are known to modify the rate at which male hormone is elaborated in the mammal. One of these—the occlusion of the excurrent canal of the testis, designated vasoligation or vasectomy—is declared to increase the amount of male hormone secreted (Steinach, '20) and is intimately connected with the problem of the origin of the internal secretion of the testis. The other factor is believed to be an influence of unknown character exerted by the female of the species; it is asserted that this influence is necessary for the maintenance of full endocrine function in the male (Steinach, '36); the subject of experimentation was the rat.

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Although these two conditions have been reported to influence hormone secretion, no thorough study of either is available in the literature. Vasectomy has been studied mainly from the point of view of whether or not it affects the spermatogenic activity of the testis; the author knows of no research reported in which quantitative tests for hormone activity are used. The report on female influence likewise contains no exact data to indicate the amount of hormone secreted in the absence of the female stimulus.

The present investigation directs special attention to these two factors alleged to modify hormone secretion in the rat, and attempts to answer the following questions: Does closure of the ductus deferens affect the rate of elaboration of testis hormone in the rat, and second, does the testicle of the rat develop and maintain hormone secreting capacity in the absence of female stimulation?

The author wishes to express her sincere thanks to Prof. Carl R. Moore for suggesting this problem and for giving unfailing encouragement and advice throughout the progress of the study. She is greatly indebted to Prof. Sewall Wright for suggesting the statistical treatment of the data.

METHODS OF PROCEDURE

Vasectomy. For the vasectomy series 216 albino rats from the stock colony of this laboratory were used. The litter was taken as a unit since it is believed that variations in size and weight in a non-inbred strain are smaller among litter mates than among non-litter mates, and because environmental conditions are known to be identical among litter mates. Each litter was divided into half experimental and half control animals all of which were kept as bachelors to obviate possible variation in the size of the accessories from different numbers of copulations.

All operations were performed in the same manner and under aseptic precautions. The incision was made through the abdomen in the mid-ventral line; the vas was brought

through with a blunt hook and the portion to be removed, midway between testis and urethra, freed from accompanying tissues. Great care was taken not to disturb spermatie blood vessels, although preliminary experimentation had indicated that it was unnecessary to dissect the vas from its blood vessels. Two fine silk ligatures were placed around the vas at a distance of at least 1.5 cm. in adult animals and correspondingly less in young rats; the intervening portion was excised. The two ligated ends were returned to the abdominal cavity and placed as far as possible from each other. If the testis had been disturbed in its scrotal position it was replaced. All operations were double vasectomies. Recovery was rapid and uneventful.

At autopsy the position of testes, completeness of vas occlusion, and presence or absence of spermatocoeles on the testicular portion of the cut vas were noted. Testes, seminal vesicles, anterior and ventral lobes of the prostate were carefully dissected free from fat and connective tissue under a binocular dissecting microscope and weighed to tenths of a milligram on a torsion balance.

Fixation of tissues was accomplished in Bouin's fluid, dehydration in alcohols or dioxane, embedding in paraffin. Ehrlich's acid hematoxylin without counterstain was used in all cases. Slides were examined for presence or absence of spermatogenesis in the testes, light areas in the ventral prostate, and secretion granules in the seminal vesicles after the method of Moore, Hughes and Gallagher ('30), and Moore, Price and Gallagher ('30).

The criterion used for determining the amount of male hormone secreted was the fresh weight of the seminal vesicles. Injection of gonadotropic substances (Moore, '36), testis extracts (Moore, Hughes and Gallagher, '30; Moore, Price and Gallagher, '30), and synthetic androgens (Moore and Price, '37, '38) into normal and castrate male rats of all ages has demonstrated that the fresh weight of the seminal vesicles varies in direct relation to the amount of male hormone present in the organism.

Isolation. In the first isolation experiment twenty-one male rats from three litters were used; the litter was taken as a unit in these experiments also. The rats were separated from the mothers at 1 month of age, and each litter was divided into experimental and control animals. The control rats were kept in the room occupied by the departmental colony. Experimental rats were placed in individual cages which were so arranged that no animal could see or come into direct contact with any other animal. These cages were kept in a closed room about 100 feet distant from that occupied by the colony. Conditions of light and temperature were approximately equal in the two rooms. Both experimental and control rats were fed the ration on which the colony is maintained. The experiment was carried on over a 10-month period.

In the second experiment a more complete isolation was attempted. A litter of ten males was weaned at 1 month of age. Each male was then placed in an individual portable cage (all cages were of the same size), and from that time on the experimental rats were never intentionally placed near another rat, male or female. Through error a hypophysectomized female was placed within 3 feet of animal 400 for 24 hours before it was discovered and removed. Three of the experimental rats were taken home by members of the department. (The writer wishes to express her appreciation of the careful attention given to these animals by Miss Ellen Moore, Mr. R. H. Denniston and Mr. C. Oldham). The other four rats were placed each in a separate room of the laboratory building as far away from each other and from the colony headquarters as was physically feasible. The three control animals were caged separately in the colony room; two were kept as bachelors and one was allowed to breed freely.

Obviously under such an arrangement conditions of light, temperature, etc., were varied, but an attempt at standardization was made by placing all of these animals on a uniform diet. A commercial ration, Purina dog chow, supplemented

by lettuce and mixed grain was given in excess of daily consumption. That this diet insures normal physiological and somatic development and is adequate for maintenance and reproduction is shown by the fact that the breeding control fathered several large healthy litters. The animals in this experiment were killed after a 7-month period of isolation.

For both isolation experiments the technique at autopsy was the same and was identical with that of the vasectomy series with one exception. Immediately preceding autopsy a receptive oestrous female was introduced into the cage of each completely isolated and each control male and permitted to remain for 5 minutes. If no attempts at mating had been made by the end of that time, the female was removed. Intromission was not permitted; as soon as it was attempted the female was withdrawn. As in the vasectomy experiments seminal vesicle weight was used as the criterion for determining the quantity of testis hormone secreted.

EXPERIMENTAL RESULTS

A. Vasectomy

Before entering into a discussion of ductus deferens closure as practiced in this series of experiments it becomes necessary to delimit the particular problem sharply. For purposes of review the literature may be divided roughly into two groups and it will be observed that the work reported here falls into the second of these groups.

The first problem to be considered is the effect of ligation upon spermatogenesis. Descriptions of cases of congenital absence or experimental section of one or both vasa deferentia in humans, rabbits, dogs and cats in which spermatogenesis was uninterrupted have appeared in the literature since 1755 when John Hunter described the anomaly in a cadaver, but in 1903 Bouin and Ancel reported interference with the spermatogenic function of the testis of rabbits and guinea pigs due to atrophic changes in the seminal epithelium after occlusion of the excurrent canal. Many authors reported conflicting results following this publication (see Oslund, '24 a,

for review). Upon the appearance of the papers of Oslund ('24), Moore and Oslund ('24), and Moore and Quick ('24), these contradictory findings appeared to be satisfactorily explained. Their work on five species of mammals showed that when the ligated testicles remained in the scrotum, no degeneration took place provided there was no circulatory interference or scrotal injury from faulty operative procedures. If artificial cryptorchidism from adhesions was a post-operative complication, the germinal epithelium degenerated. Since that time their results have been confirmed on these and four additional species of mammals by Hammond ('25), Warwick ('25), Tamura and Crewe ('26), Humphrey ('26), Cunningham ('28), Brouha and Desclin ('33), Knaus ('37), and others. Conflicting reports still appear at intervals: Sand ('33), Steinach ('36), Nakamura ('32), Gatz ('38), Wilhelm ('32), and John ('38).

The second problem associated with the results of vasoligation deals with the endocrine function of the testicle. Bouin and Ancel ('03) also found that the changes following castration did not occur following vasectomy and that the interstitial tissue of the testis did not atrophy but apparently increased in amount; thus it seemed to be indicated as the source of the testis hormone. Although this conclusion was immediately disputed and is still open to question (see Bouin and Benoit, '31 and Moore, '32) it nevertheless formed the basis for a school of thought led by Steinach. In 1920 he reported that old male rats believed from their external appearance and behavior to be senile were rejuvenated or reactivated by vasoligation and suggested that the increase in rate of testis hormone secretion was responsible for the phenomenon. Sand ('22), Wilhelm ('24), Romeis ('33), Benjamin ('22), Schmidt ('24), Ruzicka ('22), and others concur with Steinach's contentions from observations on dogs, rats, guinea pigs, cattle, horses, and humans. The rejuvenation was reported to affect not only psychic and somatic sexual characters but also other physiological phenomena such as blood count, basal metabolism, muscle tonus, nervous reac-

tions, growth of hair, optic reactions, etc., and thus opens the question of the effects of testis hormone in the organism. Since most of the work was performed before the development of quantitative tests for male hormone in the organism, little data on the characters known to be directly under the control of the hormone, such as the accessory organs of reproduction, is available, and none of the data are practicable for comparative purposes.

Reports of Price ('36) and Moore ('37) have shown that post-embryonic hormone secretion in the rat may be divided into a prepuberal period of very low hormone level lasting until 35 days of age, a second period of increased hormone secretion lasting until 70 days of age, and the adult period

TABLE 1
Numbers of animals used in vasectomy experiments

<i>Age at operation</i>	<i>Age at autopsy</i>	<i>Vasectomized</i>	<i>Normal litter mate controls</i>	<i>Total</i>
15 days	35 days	16	13	29
30 days	60 days	20	17	37
60 days	120 days	17	17	34
60 days	6 months	14	10	24
6 months	8 months	19	16	35
6 months	12 months	21	17	38
12-15 months	15-18 months	10	9	19
Total		117	99	216

in which hormone is elaborated at a constant high rate. The present experiments have been designed to cover these three phases of secretion as is indicated in table 1.

Series 1: 15 to 35 days. From table 2 it is seen that rats vasectomized at 15 days of age and sacrificed at 35 days weighed 2.57 gm. more than their controls sacrificed on the same day. The testis weight of operated rats exceeded that of the normals by 0.0312 gm.; seminal vesicle weight of operated cases increased 2% over normal. The prostate tissue does not vary in the anterior lobes but is 7.3% lighter in operated animals in the ventral lobes.

Since day 35 in this colony is the critical day for the appearance of sperm heads (a definite stage in spermatogenesis

described by Moore, '36), the testes of the rats were examined for possible inhibition or acceleration of spermatozoon development. Cross sections of the testes of operated and litter mate control animals were indistinguishable; in every operated case sperm head formation was apparent and at the same stage of development as in the normal brothers.

Histologically prostate and seminal vesicle differentiation was at the same stage in operated and control rats. Light areas were apparent in the prostate and secretion granules were characteristically lacking in the epithelium of the seminal vesicles (Price, '36).

Series 2: 30 to 60 days. In the series vasectomized at 30 days of age and killed at 60 days with their litter mate controls, the body weight of the controls was almost 4 gm. more than that of the operated (table 2); testicular weight varied in favor of the controls by 0.01 gm. Seminal vesicle weight was 12% less in the treated group, and prostate weight fluctuates differently in the anterior and ventral lobes. No detectable difference could be observed between operated and control animals in any tissue examined.

Series 3: 60 to 120 days. In the group vasectomized at 60 days of age and maintained for an equal period of time, the most noticeable effect of the operation was the appearance of spermatocoeles on the testicular ends of the severed vasa deferentia. The formation and structure of these sacs containing dead sperm and masses of cheesy material has been discussed by Leiter ('35).

Table 2 shows that the operated animals averaged 5.88 gm. lighter in body weight than their untreated litter mates, and that the testes in the experimental group weighed 0.05 gm. less than those in the control group. Seminal vesicles of the treated rats were 8% lighter than those of controls, and both portions of the prostate were slightly lighter. Histologically testes, seminal vesicles, and prostates were essentially normal in appearance in the operated group.

Series 4: 60 to 240 days. Body weight of rats vasectomized at the same age as in the preceding series but maintained for

4 months longer exceeded that of normal controls of the same age by 6 gm. The experimental testes were 0.02 gm. heavier than the normal testes, while experimental seminal vesicles were 7.5% lighter than those of control animals. Prostate weight of operated rats was slightly less than that of litter mate brothers in both portions of the gland. Histological normality was the rule in all experimental tissues. Spermatozoa were observed on all operated rats.

Series 5: 6 to 8 months. The rats operated at 6 months of age and sacrificed at 1 year exceeded their controls in body weight by 16 gm., in testis weight by 0.17 gm., in seminal vesicle weight by 18%, in coagulating gland weight by 0.0212 gm., and in ventral prostate weight by 0.638 gm. (table 2). Microscopically operated and control testes and sex accessories were indistinguishable. All operated rats exhibited spermatozoa.

Series 7: 12 months or more to 15, 16 or 18 months. In this series the limiting factor of mortality entered to such an extent that it was not possible to secure an exactly comparable set of data. The object of the series was to operate on rats as old as possible and to maintain them in the vasectomized condition for as long as possible, but after 1 year of age the death rate in this colony rises so rapidly as to invalidate many attempted experiments. Thus time of operation and of autopsy was determined empirically. Although the five litters reported are not strictly comparable in age, they have been treated as though they were. It may be stated here that there was no advantage in longevity of operated over control groups or vice versa.

From table 2 it will be observed that the operated animals were on the average 25 gm. heavier than control animals. Operated testes were 0.02 gm. lighter than control testes; the seminal vesicles of the experimental group were 9% heavier than those of the normal group. The anterior lobes of the prostate were 0.0046 gm. heavier in the vasectomized rats although the ventral lobes were 0.0096 gm. lighter.

All tissues examined showed normal spermatogenesis in the testes, secretion granules in the seminal vesicles, and light areas in the prostate in both operated and control animals. There was no evidence of reproductive senility in either group.

Statistical evaluation. To test the validity of the data presented above, Student's Method of Paired Comparisons was applied; the probability has been indicated in table 2 as p . It will be noted that with two exceptions occurring in series 3, the variation in weight between vasectomized and normal animals is insignificant if the customary value of 0.05 be taken as the upper limit of significance. The first exception occurs in the body weights of series 3; p is calculated to be 0.05, but as this is a border line case assumption of significance is unwarranted. The second exception occurs in the seminal vesicle weights of the same series. The p value of 0.02 would indicate that testis hormone secretion is inhibited when vasectomy is performed at 60 days and the animal is maintained for 2 months following the operation. The size of the variation is seen to be 8%; this undoubtedly falls within the limits of experimental error of the method. Furthermore any group of 50 p values would be expected to contain one as low as 0.02, and 40 p values are presented in conjunction with this data.

The foregoing results, then, indicate that vasectomy does not modify testis hormone secretion quantitatively as determined by histological or by gravimetric tests.

B. Isolation

In connection with the problem of psychological control of sexual development Steinach ('36) has stated that the fully developed accessory organs of reproduction in the adult male rat regress, that the testes degenerate, and that sexual drive disappears if the animal is maintained in an environment in which no female rats are present. This statement implies dependence of hormone secretion of the testis upon a stimulus furnished by the female. Steinach postulates that this stimulus

enters the brain through the olfactory tracts; in the brain an unexplained mechanism stimulates the sympathetic apparatus controlling the vasomotor system of the genital tract, and produces a hyperemia of the reproductive organs. Through this hyperemia a larger quantity of the gonadotropic principle of the anterior lobe of the hypophysis is made available to the male hormone producing tissues, and with the increased stimulus an increased amount of hormone is secreted.

This hypothesis is somewhat difficult to accept in view of the work of Stone ('23) who demonstrated in the rat that the elimination of visual, olfactory, and gustatory sense functions in young males prior to sexual experience and in sexually experienced adults produces no changes in the sexual act, either in the essential pattern, the age at which it appears, or the responsiveness of a male to a receptive female. Later ('25) he found that transection and destruction of the olfactory bulbs did not markedly affect the sexual behavior of male rabbits. Von Bechtrew ('11) observed no changes in the sexual behavior of anosmic dogs. Magnotti ('36), on the contrary, found that guinea pigs of both sexes whose olfactory lobes had been transected prepuberally failed to copulate and that upon histological examination their reproductive systems showed inhibition of development.

Stone ('26) reported that no factors of environment beyond those necessary for normal somatic development are required to bring about sexual maturity in the female rat as evidenced by ability to perform the copulatory act during the receptive phase of oestrous. These females were individually caged but it is not stated that they were isolated spatially from males. Slonaker ('36) noted that male rats in revolving cages showed increased activity when oestrous females were brought within a distance of 45 cm.; a greater distance provoked no response.

Repetition of Steinach's experiments with some modifications gave the following results. As is seen in table 3 the body weight of the rats isolated only from females was 12.71 gm. less than that of their controls. The testis weight

of these experimental rats was 0.03 gm. more than that of their normal litter mates. Seminal vesicles of the isolated group exceeded those of the non-isolated by 0.15%; the prostate in both anterior and ventral lobes was slightly smaller in the isolated rats. Application of Student's Method of Paired Comparisons shows these differences to be insignificant since the value of p is never less than 0.05.

TABLE 3

Mean weights, mean differences (D), and probability (p) of organs of eleven male rats isolated from females at 1 month of age and sacrificed with eight littermate controls at 11 months of age

	<i>Isolated</i>	<i>Control</i>	<i>D</i>	<i>p</i>
Body weight, grams	307.96	321.33	—12.71	0.4
Testes, grams	2.96	2.93	+0.03	0.9
Seminal vesicles, grams	0.8673	0.8660	+0.0013	0.9
Coagulating glands, grams	0.1307	0.1596	—0.0285	0.3
Ventral prostate, grams	0.4387	0.5443	—0.1052	0.4

TABLE 4

Mean weights and standard errors of organs of six male rats isolated from male and female rats from 1 month of age and sacrificed with three litter mate controls at 8 months of age

	<i>Isolated</i>		<i>Control</i>	
Body weight, grams	340	± 10	313	± 7.24
Testes, grams	2.94	± 0.12	2.49	± 0.13
Seminal vesicles, grams	1.1812	± 0.0300	1.1577	± 0.1208
Coagulating glands, grams	0.1487	± 0.0255	0.2013	± 0.0287
Ventral prostate, grams	0.4595	± 0.0234	0.4954	± 0.0420

In the experiment in which the rats were completely isolated, from table 4 it can be seen that the isolated animals weighed 27 gm. more than their controls; that their testes were 0.44 gm. heavier; that their seminal vesicles were 0.0235 gm. heavier or 2%, and that the prostates were lighter in both lobes. These differences are not beyond the limits of normal variation and are, therefore, not significant.

Upon histological examination the testes and sex accessories of control rats were found to be indistinguishable from those of isolated rats in both experiments.

Tests for the presence of sexual drive conducted upon the rats of the completely isolated group indicated that desire for copulation was as evident in the isolated rats as in the control rats with a single exception. Animal 400—the one accidentally exposed to the presence of a hypophysectomized female for 24 hours—showed no interest of sexual character in the oestrous female placed in its cage although this same female had been pursued by animal 404 a half hour previously.

From these observations it may be stated that the isolation of male rats from female rats for a period from immediately after weaning to the age of 11 months had no perceptible effect upon the development of the testis or other accessory organs of reproduction, upon the quantity of male hormone secreted as indicated by the fresh weights of the organs or the histological appearance of the tissues. The same is true of individual males maintained in a home where no other rat was present during a period of 7 months and is further borne out by tests for sexual drive.

DISCUSSION

As indicated earlier in this paper investigations on the effects of vasectomy aside from an effective sterilization procedure have been directed to two principal problems: the effects on spermatogenesis, and the effects on testis hormone secretion. Special attention has been directed here to the latter of these problems due to the fact that the literature contains no satisfactory record of enquiry into the problem but is replete with suggestions of increase in testis hormone secretion following such a surgical procedure.

Considering first and briefly the possible influence on spermatogenesis attention is called to the review of Moore ('39) in which there are presented extensive citations of observations following closure of the vas deferens in many different species of mammals and classes of vertebrates. He has emphasized especially the negative contention. There may be added to his references on mammals a few further citations.

Gatz ('38) has stated that the testes of vasoligated rats decrease in size about 50% after maintenance for 2 months in the vasoligated condition. Such a decrease is not apparent in the testicular weights of the rats of the present study. Although he states that the testes were scrotal in position in all cases, he reports an inconsistent histological picture; in some cases the testicle was degenerate, in other cases normal. The possibilities of operative injury, infection, or interference with vascularization or innervation are not mentioned; he does not state whether the operative approach was through the abdomen or the scrotum. It has been observed by Moore and Quick ('24) that scrotal incisions are more apt to result in injuries to the testicle than abdominal incisions. With regard to the author's operations it may be remarked that while all approaches were abdominal, following the earlier operations atrophic and cryptorchid testes were found more often on autopsy than were observed in the later procedures. This indicates more efficient avoidance of troublesome consequences of a purely surgical nature as one's experiences increase.

Leiter ('36) reports no changes in spermatogenic activity of the testes of the rat after interruption of the vas deferens for periods of up to 1 year. Brouha and Desclin ('33) failed to find marked histological changes in either the cells of the tubules or the interstitial tissue of the testis after section of the vas lasting from 4 to 7 months in rats and 13 months in rabbits. Knaus ('37) found no cessation of spermatogenesis in the rabbit after 1 year of vasectomy; sperm from the epididymides of three of these animals was used to fertilize six females successfully. Haberland ('35) found no effect of vasoligation in thirty-seven guinea pigs when the operation was performed at the age of 10 to 14 days or 3 months. Nakamura ('32) found degeneration of the seminal epithelium and hyperplasia of the interstitial cells in rabbits after vasectomy but there seems to be little doubt that interference with vascularization was a corollary.

The main criticism of the contradictory results of American workers on vasoligation, by the European school, has been voiced by Sand ('33) who maintains that the animals are kept for an insufficient length of time after the operation for the effects to become evident. In reply to such objections attention is called to the fact that in two series of the author's experiments rats were operated and left undisturbed for 6 months. As has been indicated earlier the average life span of a rat in this colony is believed to be under 2 years; an effect of the operation would surely be expected to occur during one-quarter of the life span of the animal. On page 2154 Sand ('33) states that the observations of Moore and Quick ('24) which pertained to animals vasoligated for a 6-month period were too short, but on page 2152 figure 51 reproduces a picture from Bouin and Ancel. This is a picture of a rabbit testis which had been vasoligated for but 1 month and shows complete germinal epithelium degeneration. In corroboration of the histological normality of the vasoligated testes of the present report, it may be said that epididymal smears made of every operated adult animal contained motile spermatozoa in every case in which the testis was uninjured by the operative procedure.

From results presented from the series of 117 operated cases reported herein involving rats from prepuberal age to the age of more than 1 year, it becomes apparent that contentions of germinal epithelium degeneration are no longer tenable. This fact coupled with the lack of acceptable evidence for interstitial cell hypertrophy or for increased hormone secretion following any procedure in which 'apparent' interstitial cell increase may have occurred would remove the basis of suggestion of greater hormone secretion upon which the idea was based.

The results obtained here on vasectomy are not strictly comparable to those of other workers since contentions with regard to testis hormone secretion have involved chiefly so-called senile animals in connection with rejuvenation. Presumably no senile rats have been used in these experiments;

distinguishing features of this intangible entity can be only arbitrary. Wiesner ('32) showed that external appearance is not a reliable indicator of sexual senescence. He described rats whose external appearance was one of marked old age but which were found to have large accessory reproductive organs; conversely, rats of equal age whose appearance was healthy and vigorous were observed to have very small sex accessories. Until it becomes possible to distinguish in the intact animal between changes of senescence and changes of organic or functional pathology, it seems at best a subjective opinion to call an animal 'senile.' It appears probable, however, that if vasectomy alters the rate of hormone production in the aged rat, some similar effect of the operation should be evidenced in the normal young or adult animal.

The criteria used by other workers for judging the rate of male hormone secretion are not entirely satisfactory for comparative purposes. Photographs and drawings of the accessory reproductive organs of 'senile' and 'reactivated' rats unaccompanied by objective data on the obvious size variation are presented by Steinach ('20, '36). The enormous variation in seminal vesicle size in normal litter mates must be kept in mind in evaluating such exhibits. When size has been shown to be capable of measurement the presentation of subjective evidence alone is unconvincing.

This type of evidence apparently has been satisfactory for many authors; they have ligated the excurrent canal of the testis, observed degeneration, assumed interstitial cell hypertrophy and increased testis hormone secretion, and proceeded to attribute other observed post-operative effects to this supposed increase in rate of secretion. These other post-operative effects are extremely varied in nature. Wilhelm ('24) reported an increase in the number of red and white blood cells in senile dogs following vasoligation; Steinach ('36) reports the same finding in senile rats. In the same paper he claims a general hyperemia of the brain and musculature leading to an increase in muscle strength and contractility; the same findings had been reported by Wilhelm earlier ('30).

In 1930 Wilhelm asserted that the lipoid pigmentation of the spinal ganglion cells which is usually found during old age disappeared after ductus deferens closure in old rats, dogs and humans. Ruzicka ('22) affirmed the reversal of protoplasmic hysteresis in rats reactivated by vasoligation. Sand ('22), Benjamin ('22), Schmidt ('24) report lowering of high blood pressure in man as a consequence of the operation. These and many other beneficial effects claimed by various investigators are believed by them to be the direct result of a rise in the amount of testis hormone available in the organism as is shown by the fact that injection of testis extracts is also reported in the same papers to produce these effects.

The conclusions of the present author obviously do not confirm or deny these findings reported as sequelae of vasectomy; the question of the effects of testis hormone in the organism as a whole will not be discussed. It is to be emphasized, however, that characters known without question to be directly under the control of the internal secretion of the testis—the size of the prostate and seminal vesicles—are not affected by vasectomy within the limits of accuracy of the index employed, and thus there is no objective evidence that vasectomy appreciably modifies the amount of hormone secreted. Since no degeneration of the germinal epithelium and no hypertrophy of the interstitial cells was observed, the premises upon which the rationale of vasectomy as practiced by Steinach and his adherents is based are not upheld. The alleged effects of the operation upon 'senile' animals are yet to be explained; at the moment it can only be repeated that what effects there may be cannot be attributed to the postulated increase in testis hormone secretion.

With respect to the 'psychological influences' on hormone secretion, or the possible influence of closely associated females, the results reported herein contrast markedly with those of Steinach ('36). Before entering into a discussion of the dissimilarity, it is perhaps advisable to list the points wherein his experimental attack on the problem differed from that of the present investigator.

1. The animals used by Steinach were not individually caged although no cage contained more than four animals; thus unisexual contact was possible.

2. No mention is made of any normal control animals.

3. The rats were subjected to exploratory laparotomy after 4 months of isolation to insure that the sex accessories had reached a normal state of development. At this time they were permitted access to oestrous females for a brief period to determine whether or not sexual drive was present.

4. At month intervals thereafter receptive females were introduced into the cages and allowed to remain for a length of time sufficient to determine the presence or absence of sexual drive.

5. After the end of the isolation period of 8 months an unspecified number of males were sacrificed while the others were returned to the presence of females for 1 month before autopsy. These males were blinded.

6. Apparently the criterion for measuring the size of the sex accessories was the subjective visual judgment of the operator.

In evaluating the results of the present report it is first necessary to call attention to the fact that no normal, unisolated, control rats are mentioned by Steinach. It must be assumed that the animals returned to the presence of females after the isolation period were considered as control animals. In view of the normal variation in the weights of sex accessories in the normal untreated litter of non-inbred stock, this type of control is questionable. Whether or not the stock used in Steinach's experiments is an inbred stock is not reported.

The second point of interest is that no statements regarding conditions of light, temperature, food, or other environmental factors are made, and in this type of experiment such factors may play an important role. In the third place the wisdom of exploratory laparotomy may be questioned; while operative injuries are improbable, the possibility of their occurrence cannot be totally withheld from consideration.

The final point and one most necessary of emphasis is that the criterion for determination of sex accessory size was a

subjective one and one which does not consider individual variation. As in the rejuvenation papers photographs of sex accessories are presented as the sole objective evidence. While there is an unquestionable size difference in favor of the non-isolated rat, an actual case from the author's group of animals may serve to illustrate the need for specific data. Two control litter mate rats from the first isolation experiment were found to vary 40% in seminal vesicle weight; the seminal vesicles of rat 207 weighed 1.1020 gm. and those of rat 208 weighed but 0.7830 gm. With such a variation in normal animals it is difficult to place confidence in photographs alone.

Apparently a more complete isolation was maintained in the present experiments. The rats used were individually caged so that contact between rats of the same sex was impossible. Experimental animals were completely isolated from females throughout life in both groups, and in one group they were isolated from males as well. None of the animals were subjected to surgical interference. Normal litter mate controls were kept in the colony room. If an effect of isolation on the rate of hormone secretion was to be observed, it seems that the more rigorous conditions of these experiments would surely have emphasized the effect. In view of these facts it appears justifiable to conclude that no stimulus from another female or male is required to develop or maintain the normal internal secretory capacity of the testicle.

SUMMARY AND CONCLUSIONS

1. Upon histological examination testes of 117 rats vasectomized at ages varying from 15 days to 15 months and maintained in the operated condition for periods ranging from 20 days to 6 months failed to show degeneration of the germinal epithelium or hypertrophy of the interstitial cells if the operation was uncomplicated by adhesions or interference with vascularization.

2. No acceleration or inhibition of spermatogenesis was observed in 35-day-old rats which had been vasectomized for 20 days.

3. In every operated case above the age of 120 days in which the testis was uninjured, spermatocoeles occurred on the testicular end of the severed vas deferens.

4. When weights of seminal vesicles and other accessory sex organs of these 117 rats were compared with those of their ninety-nine normal litter mate controls kept in the same environment, no significant variation in weights was observed.

5. Since seminal vesicle weight in the rat is an indicator of the amount of testis hormone present, it is shown that vasectomy does not modify the rate of male hormone secretion.

6. Male rats when placed in separate cages spatially isolated from female rats from the age of weaning (30 days) until autopsy at the age of 11 months showed no significant difference from their litter mate controls in the weights or histological appearance of the testes and accessory organs of reproduction.

7. Seven litter mate male rats caged individually at the age of weaning were completely isolated from all other rats for 7 months; they did not vary significantly in tests for presence or absence of sexual drive or in weights and histological appearance of testes and sex accessories from the three individually caged normal litter mate brothers kept in the colony room.

8. It is concluded on the basis of these experiments that testis hormone secretion in the male rat is not modified when the animal is isolated from male influence or from female influence or from both.

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